Immunology 1991 **72** 440–442

ADONIS 0019280591000745
BRIEF COMMUNICATION

Inhibition of tumour necrosis factor alpha (TNF-α)-induced neutrophil respiratory burst by a TNF inhibitor

A. FERRANTE,* B. HAUPTMANN, P. SECKINGER & J.-M. DAYER *Division of Immunology and Allergy (Hans Wilsdorf Laboratory), Hospital Cantonal University of Geneva, Geneva, Switzerland*

Accepted for publication 20 November 1990

SUMMARY

Tumour necrosis factor alpha (TNF-α) plays an important role in microbial defence and tissue damage by activating neutrophils. Therefore the ability of natural molecules to regulate the activity of TNF-α is likely to be of major importance in our understanding of the mechanisms of inflammation. We have examined the effects of a highly purified urine-derived TNF inhibitor (TNF inh) on the TNF-α-induced respiratory burst in human neutrophils. TNF-α inh-treated TNF-α was unable to stimulate a neutrophil lucigenin-dependent chemiluminescence response and superoxide formation. Treatment of TNF with the inhibitor also significantly reduced the priming ability of TNF-α for a response to the peptide f-met-leu-phe. These results show that the ability of TNF-α to induce a key neutrophil response is amenable to regulation by the TNF-α inh.

The cytokine tumour necrosis factor-alpha (TNF-α) has pleiotropic effects, playing a role in pathophysiological mechanisms in a variety of diseases, such as septicaemia and in cachexia, and in the regulation of the inflammatory response.[1–7] Recently molecules which regulate the activity of TNF-α have been described. These were found in high concentrations in the urine of febrile patients.[8] One of these inhibitors has been shown to inhibit TNF-α-mediated tumour cell cytotoxicity, TNF-α-induced prostaglandin E_2 production by dermal fibroblasts, TNF-α-induced class I expression in the Colo 205 tumour cell line and the synergism between TNF-α and interferon-gamma (IFN-γ)-induced HLA-DR antigen expression.[9–11] The TNF inhibitor (inh) is a 31,000–33,000 molecular weight (MW) protein with a PI of 5·5–6·1.[9] The mechanism of its action is to bind tightly to TNF and prevent TNF from binding to its receptor.[10] This inhibitor is now considered to be a soluble form of the TNF receptor.[12,13] TNF-α is a neutrophil activator and primes neutrophils for increased microbial killing and tissue damage.[4,14,15] We therefore examined whether or not this TNF-α inhibitor prevented neutrophil activation by TNF-α.

Neutrophils were prepared from blood of healthy volunteers by the rapid single-step method of Ferrante & Thong[16] using Hypaque–Ficoll medium (monopoly resolving medium; Flow Laboratories, Zurich, Switzerland). The neutrophils were of >96% purity and >99% viability. Recombinant human TNF-α was produced in *Escherichai coli* (Biogen S.A., Geneva, Switzerland). Urine-derived TNF-α inh was purified to homogeneity by sequentially TNF-α affinity column, Mono-S cation-exchange and reverse-phase chromatographies.[11] The specific activity of the TNF-α inhibitor was $1{\cdot}38 \times 10^6$ units/mg in which one unit of activity was defined as the minimal quantity of inhibitor required to inhibit 50% of 0·2 ng/ml (specific activity $9{\cdot}6 \times 10^8$ U/mg) of TNF-α-induced cytotoxicity in the presence of actinomycin D.[11]

The respiratory burst activity was examined by the lucigenin-dependent chemiluminescence assay and the cytochrome C reduction assay.[17–19] In the former, 40 μl of TNF-α (40 ng) were treated with 40 μl of TNF-α inh for 15 min and then examined for ability to stimulate neutrophil chemiluminescence by adding the TNF-α–TNF-α inh mixture to 100 μl of 1×10^7 neutrophils/ml. The volume was brought to 500 μl with Hanks' balanced salt solution (HBSS) and 500 μl of 200 μg/ml of lucigenin added. The tubes were placed in a 37° water jacketed incubator in a luminometer (LKB, Wallac, Finland) and the light emitted measured in mV. The results are presented as the peak rate of chemiluminescence production, unless specified otherwise. In the cytochrome C reduction assay, 10 μl of TNF-α (10 ng) were mixed with 10 μl of TNF-α inh and then 20 μl of superoxide dismutase (SOD) and 200 μl of cytochrome C added. The volume was brought to 1 ml with HBSS. A parallel set of tubes were set up without SOD. The tubes were incubated for 30 min, centrifuged and the OD_{515} read in a spectrophotometer. The superoxide produced was determined from the difference in OD of the respective samples with and without SOD. In some studies the effects of the tripeptide, f-met-leu-phe (FMLP) were examined (1×10^{-6} M). The data presented were analysed by the two-tailed *t*-test.

TNF-α induced a chemiluminescence response in neutrophils, approximately 24-fold above the basal level ($P < 0{\cdot}01$) (Figs 1 and 2). Peak chemiluminescence was seen at approximately 10 min (Fig. 2). Treatment of TNF-α with the inhibitor

* Present address and correspondence: Dept. of Immunology, Adelaide Children's Hospital, South Australia 5006, Australia.

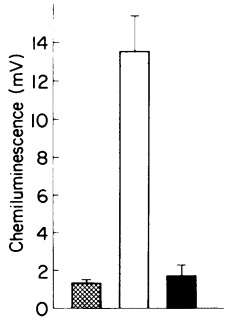

Figure 1. The effect of TNF inh on the TNF-α-induced neutrophil chemiluminesce response. Neutrophils were either not treated (▩), treated with TNF-α (□) or treated with TNF inh–TNF-α (■).

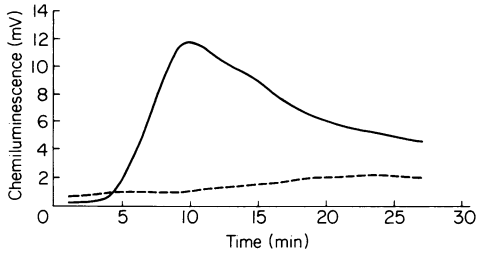

Figure 2. Time-related changes in rate of chemiluminescence production by neutrophils treated with TNF-α (——) or TNF inh–TNF-α (– – – –).

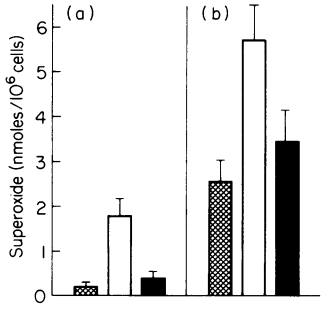

Figure 3. The effects of TNF inh on superoxide production by neutrophils treated with TNF-α (a) or primed with TNF-α for an FMLP response (b). (a) Non-treated (▩), TNF-α (□), TNF inh–TNF-α (■). (b) FMLP (▩), TNF-α-primed + FMLP (□), TNF inh–TNF-α-primed + FMLP (■).

completely abolished its ability to stimulate a chemiluminescence response ($P < 0.01$). Studies in which superoxide was measured by the cytochrome C system confirmed these findings (Fig. 3). TNF-α induced a superoxide response ($P < 0.05$) and primed neutrophils for an augmented response to FMLP ($P < 0.05$). In both cases the effects were abolished by TNF inh ($P < 0.05$). The inhibitor was found not to have any significant effect on the FMLP-induced response in the absence of TNF. In three experimental runs the neutrophil response to TNF-α inh-treated FMLP was similar to that induced by FMLP alone (95 ± 25% of control response).

The data presented that the TNF inh can prevent TNF-α from activating neutrophils. The inhibitor was specific in its action. It had no effect on the basal respiratory activity of neutrophils and it did not affect the response to FMLP, but did affect the TNF-α priming for an FMLP response. Similar specificity has already been observed previously in that the TNF inh was ineffective in inhibiting the LAF activity of interleukin-1 (IL-1)[11] and the INF-γ-induced HLA-DR expression on the human Colo 205 tumour cell line.[11] Evidence is presented which shows that both the TNF-α-induced chemiluminescence response and superoxide production are inhibited by TNF inh. This neutrophil activity is a measure of the production of oxygen-derived reactive species such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen which have been implicated in both microbial killing and tissue damage.[14,20] TNF-α can either induce a respiratory burst in neutrophils or prime the cells for an increased response to other stimuli. The latter is not only relevant to FMLP but also to opsonized bacteria,[4] fungi,[19] amoebae,[18] tumour cell killing[2] and tissue damage.[20] Thus the ability to regulate this function by natural inhibitors may have important implications in the understanding of the mechanisms of inflammation.

There exist two forms of TNF binding proteins consistent with the presence of two receptors for TNF, a 55,000 MW and 75,000 MW protein.[21-23] The present data suggest that the inhibitor prevents binding of TNF to both types of TNF-binding proteins on neutrophils since it was capable of totally blocking the response. While the TNF-α inh may be found in normal urine and serum,[8,24] it is evident that appreciable levels are associated with fever.[8,25] Other sources include synovial fluid[26] and alveolar macrophages.[27] However, to date little is known of the type of stimuli and the conditions under which the TNF-α receptor binding protein is released. Some recent studies[28] have shown that the TNF receptor is shed by activated human neutrophils and is similar in MW (approximately 28,000) to the TNF inh studied in our present investigation. Results from our study suggest that the TNF–TNF inh complex is unable to stimulate neutrophils and in this manner neutrophils probably protect themselves from TNF-induced damage. This would be in addition to the findings that TNF receptors on neutrophils are down-regulated by TNF itself as well as various agonists.[6] These observations may also explain why in our previous investigations we found that if TNF was added after rather than prior to a stimulus such as FMLP or opsonized fungi, the cytokine failed to stimulate the response.[19,20]

ACKNOWLEDGMENTS

This work was supported by grants from the Swiss National Science Foundation (Grant No. 31.26424-89), The Foundation Elsie Carlos de Reuter Medical Center, the Australian National Healthand Medical Research Council and UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

REFERENCES

1. Arend W.P. & Dayer J.-M. (1990) Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. *Arthr. Rhem.* **33,** 305.
2. Balkwill F.R. & Burke F. (1989) The cytokine network. *Immunol. Today*, **10,** 299.
3. Brenner M.K. (1988) Annotation: tumour necrosis factor. *Br. J. Haematol.* **69,** 149.
4. Ferrante A. (1990) Activation of neutrophils by interleukin 1 and 2 and tumour necrosis factors. In: *Granulocyte Responses to Cyto-*

kines: Basic and Clinical Research (ed. R. G. Coffey). Marcel Dekker, New York (in press).

5. PLAYFAIR J.H.L., TAVERNE J., BATE C.A.W. & DE SOUZA J.B. (1990) The malaria vaccine: anti-parasite or anti-disease? *Immunol. Today*, **11,** 25.
6. SCHLIFFEBNAUM B. & FEHR J. (1990) The tumour necrosis factor receptor and human neutrophil function. Deactivation and cross-deactivation of tumour necrosis factor-induced neutrophil responses by receptor down-regulation. *J. clin. Invest.* **86,** 184.
7. TRACEY K.J., LOWRY S.F. & CERAMI A. (1988) Cachectin: a hormone that triggers acute shock and chronic cachexia. *J. infect. Dis.* **157,** 413.
8. DAYER J.-M. (1990) Natural inhibitors of interleukin-1 and tumour necrosis factor α. *Finst Jenner Symposium*, John Wiley & Sons (in press).
9. SECKINGER P., ISAAZ S. & DAYER J.-M. (1988) A human inhibitor of tumour necrosis factor α. *J. exp. Med.* **167,** 1511.
10. SECKINGER P., ISAAZ S. & DAYER J.-M. (1989) Purification and biologic characterization of a specific tumour necrosis factor α inhibitor. *J. Biol. Chem.* **264,** 11966.
11. SECKINGER P., VEY E., TURCATTI G., WINGFIELD P. & DAYER J.-M. (1990) Tumour necrosis factor inhibitor: purification, NH_2-terminal amino acid sequence and evidence for anti-inflammatory and immunomodulatory activities. *Eur. J. Immunol.* **20,** 1167.
12. ENGELMANN H., NOVICK D. & WALLACH D. (1990) Two tumour necrosis factor binding proteins purified from human urine *J. biol. Chem.* **265,** 1531.
13. SECKINGER P., ZHANG J.-H., HAUPTMANN B. & DAYER J.-M. (1990b) Characterization of a TNF-α inhibitor. Evidence of immunological cross-reactivity with the TNF receptor. *Proc. natl. Acad. Sci. U.S.A.* **87,** 5188.
14. FERRANTE A., KOWANKO I.C. & BATES E.J. (1990) Mechanisms of host tissue damage by neutrophils activated by cytokines. In: *Granulocyte Responses to Cytokines: Basic and Clinical Research* (ed. R. G. Coffey). Marcel Dekker, New York (in press).
15. STEINBECK J.J. & ROTH J.A. (1989) Neutrophil activation by recombinant cytokines. *Rev. Infect. Dis.* **11,** 549.
16. FERRANTE A. & THONG Y.H. (1982) Separation of mononuclear and polymorphonuclear leucocytes from human blood by the one-step hypaque-ficoll method is dependent on blood column height. *J. Immunol. Meth.* **48,** 81.
17. BABIOR B.M., KIPNES R.S. & CURNUTTE J. (1973) Biological defence mechanisms: the production by leukocytes of superoxide, a potential bactericidal agent. *J. clin. Invest.* **52,** 741.
18. FERRANTE A. (1989) Augmentation of neutrophil responses to *Naegleria fowleri* by tumour necrosis factor alpha. *Infect. Immun.* **57,** 3110.
19. FERRANTE A. (1989) Tumour necrosis factor alpha potentiates neutrophil antimicrobial activity: increased fungicidal activity against *Torulopsis glabrata* and *Candida albicans* and associated increase in oxygen radical production and lysosymal enzyme release. *Infect. Immun.* **57,** 2115.
20. SEGAL A.W. (1989) Perspectives: the electron transport chain of the microbicidal oxidase of phagocytic cells and its involvement in the molecular pathology of chronic granulomatous disease. *J. clin. Invest.* **83,** 1785.
21. BROCKHAUS M., SCHOENFELD H.-J., SCHLAEGER E.-J., HUNZIKER W., LESSLAVER W. & LOETSCHER H. (1990) Identification of two types of tumour necrosis factor receptors on human cell lines by monoclonal antibodies. *Proc. natl. Acad. Sci. U.S.A.* **87,** 3127.
22. HOHMANN H.-P., REMY R., BROCKHAUS M. & VAN IOON A.P.G.M. (1989) Two different cell types have diffrent major receptors for human tumour necrosis factor (TNFα). *J. biol. Chem.* **264,** 14927.
23. LOETSCHER H., PAN Y.-C., LAHM H.-W., GENTZ R., BROCKHAUS M., TABUCHI H. & LESSLAUER W. (1990) Molecular cloning and expression of the complete human 55 kd tumour necrosis factor receptor. *Cell*, **61,** 351.
24. ENGELMANN H., ADERKA D., RUBINSTEIN M., ROTMAN D. & WALLACH D. (1989) A tumour necrosis factor-binding protein purified to homogeneity from human urine protects cells from tumour necrosis factor toxicity. *J. biol. Chem.* **264,** 11974.
25. PEETRE C., THYSELL H., GRUBB A. & OLSSON I. (1988) A tumour necrosis factor binding protein is present in human biological fluids. *Eur. J. Haematol.* **41,** 414.
26. ROUX-LOMBARD P., MODOUX C. & DAYER J.-M. (1988) Inhibitors of IL-1 and TNFα activities in synovial fluids and cultured synovial cell supernatants. *Calcif. Tissue Int.* **22** (Suppl.) A47.
27. DE ROCHEMONTEIX-GALVE B., DAYER J.-M. & JUNOD A.F. (1990) Fibroblast-alveolar cell interactions in sarcoidosis and idiopathic pulmonary fibrosis: evidence for stimulatory and inhibitory cytokine production by alveolar cells. *Eur. Respir. J.* **3,** 653.
28. PORTEU F. & NATHAN C. (1990) Shedding of tumour necrosis factor receptors by activated human neutrophils. *J. exp. Med.* **172,** 599.
29. FERRANTE A., NANDOSKAR M., WALZ A., GOH D.H.B. & KOWANKO I.C. (1988) Effects of tumour necrosis factor alpha and interleukin-1 alpha and beta on human neutrophil migration, respiratory burst and degranulation. *Int. Arch. Allergy appl. Immunol.* **86,** 82.